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THE EFFECT OF LASER HARDENING AND EMBEDDING NANOPARTICLES ON TOOTH RESISTANCE AGAINST CARIES

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ABSTRACT

Tooth caries begin when acids formed by bacteria in the plaque penetrate the dentin and dissolute its minerals. Lasers illumination can improve dental enamel properties against demineralization. Tooth resistance to mineral loss can be improved by combining the effect of laser with re-mineralizing nanoparticles. Thirty two extracted teeth in good dental health were used in this study. Q-switched Nd: YAG laser was utilized to illuminate the sectioned teeth samples; immersed in nano-colloidal silver solution. Each sample was treated by the same laser energy fluence but with different number of laser pulses. Vickers Micro-hardness tester, Atomic Force Microscope AFM, scanning electron microscope SEM, and Energy dispersive X-ray spectrometer were employed to characterize the treated samples. The samples treated with laser alone showed moderate increased hardness, but a higher hardness value was demonstrated when treated with combined laser and silver-nanoparticles. The treated samples revealed significant changes in the topography and granularity distribution; with a notable increase in enamel surface roughness due to laser induced-crystallographic changes. The enamel grain size and structure showed significant changes after treatment. The demineralized samples of the laser treated samples demonstrated higher Ca/P ratios than the demineralized control samples; which meant better acid resistance.

KEYWORDS: Nd: YAG Laser, Silver Nanoparticles, Tooth Resistance, De-Mineralization & Laser Hardening

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Original Article

INTRODUCTION

Tooth decay begins when pH reduces below 5.5 [1], acids formed by bacteria in the plaque penetrates the dentin and dissolute its minerals [2, 3]. Although fluoride is the most powerful treatment to prevent tooth decay, it is still necessary to develop new methods to entirely control this disease. Lasers have been tested to improve dental enamel properties in order to enhance its resistance to demineralization [4].The expansion of the anti-caries factors can diminish caries rates and increase dental protection. Tooth resistance to mineral loss can be maintained by combining the effect of laser with re-mineralizing nanoparticles [5]. Ruby laser irradiation (wavelength 693 nm) was first suggested in 1972 to prevent dental caries [6]. This in vitro study demonstrated an increase in the resistance to subsurface demineralization of human dental enamel exposed to the laser [7]. The use of this laser was later on excluded when treated teeth demonstrated crater-like morphologies [8]. Careful adjustment of laser parameters is so important to avoid damaging neighbouring tissues of tooth [9]. Short pulsed Nd: YAG laser was recently proposed to treat dental hard tissues because of the very short thermal diffusion time [10]. In 1989 one research team [11] used 30 J/cm^2 pulsed Nd: YAG laser (1064 nm) with fluoride group and found a decrease of the dissolved calcium by 90% [12, 13]. The effect of physicochemical changes in dental enamel proved the growing

resistance to demineralization [14]. In 2000, a research group reported a 15% decrease in tooth demineralization after laser irradiating the tooth [15]. In 2007, a combined topical fluoridation with 5 Watt and 7 Watt diode lasers was used in an in-vitro study on the enamel surface. The study revealed some changes in the enamel surface and a damage prevention of the enamel acid attack [16].The aim of the present work is to evaluate the effect of Nd: YAG and Silver nanoparticles on decreasing dental caries; by increasing the enamel hardness, decreasing the enamel solubility, and expelling the bacteria from the enamel surface.

EXPERIMENTS

Thirty two extracted teeth in good dental health with no active decay were washed and brushed with toothpaste. The teeth were then put in plastic molds, and an epoxy resin was poured in the molds with the tooth crown exposed. These molds were lifted after the epoxy became solid. The teeth were then sectioned using a low-speed diamond wheel saw under water cooling. The sectioned samples were polished using grinding machine and Amery silicon carbide papers under water spray cooling; figure (1). A cloth and 0.05 polishing alumina were used to as a final polishing stage. The samples were then cleaned ultrasonically in distilled water for 5 minutes and stored in distilled water with 10% formalin at room temperature. Q-switched Nd: YAG laser (HUAFEL, Single Pulse Mode – China), that provides up to 1 Joule, 9 ns laser pulses at 1.06 µ wavelength was utilized to illuminate the sectioned teeth samples. A converging lens of 10 cm focal length lens was used to control the laser energy fluence on the tooth samples. A light-yellow transparent Nano-colloidal silver solution was prepared by reducing 4 mL of 0.001M silver nitrate ($AgNO_3$) into 60 mL of 0.002M sodium borohydride ($NaBH_4$).

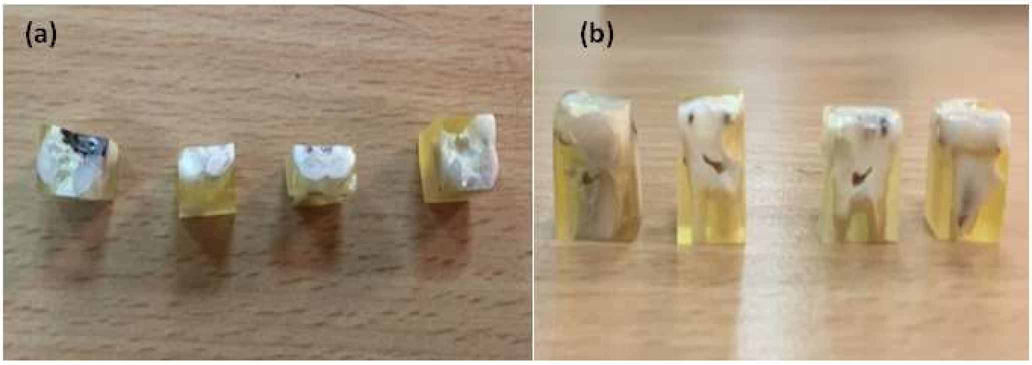

Figure 1: Teeth Samples: (a) Top View, (b) Side View

The UV- Visible spectrometer (UV-1800, Shimadzu Corporation, Japan) absorption result of the silver colloidal solution is presented in figure (2). An average silver nanoparticle size of 51nm was obtained from the Atomic Force Microscope (SPM-AA3000, AFM- Contact mode, Angstrom Advanced INC.USA) as shown in figure (3). According to the scanning electron microscope image, the silver nanoparticle size was approximately 100nm because of the particles aggregation as shown in Figure (3.11).

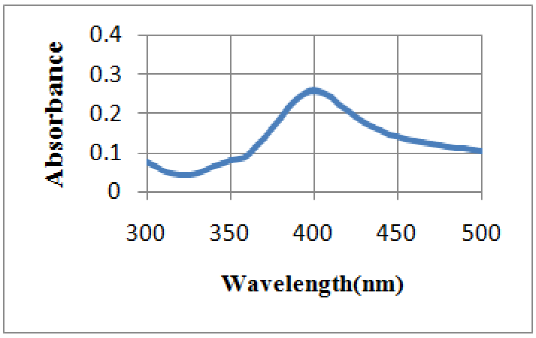

Figure 2: The Absorption Spectrum of AgNPs

The tooth samples were divided into six subgroups; each comprises 3 samples. Sub-group (A) is the control untreated samples. Each subgroup was treated by the same laser energy fluence but with different number of laser pulses.

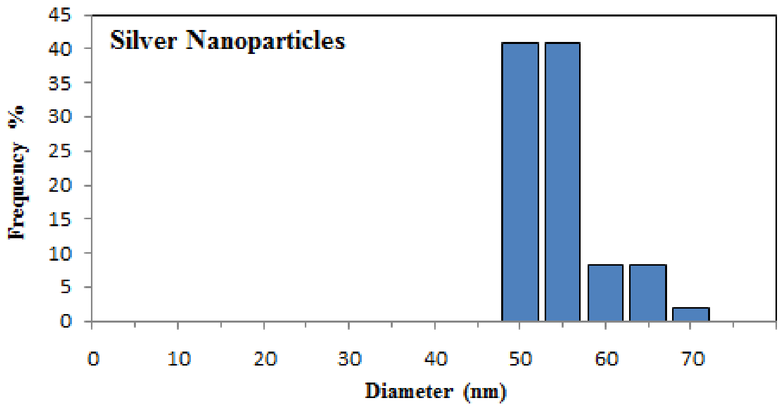

Figure 3: Granularity Accumulation Distribution Chart of AgNPs

Vickers Micro-hardness test was conducted to study the tooth surface hardness changes after laser illumination. TH 715, 2008 Vickers hardness machine, China; equipped with a high-resolution optical microscope was utilized. SPM-AA3000, AFM - Contact mode, Angstrom Advanced INC. USA; Atomic Force Microscope was employed to study the surface roughness of the tooth enamel samples; before and after laser irradiation. Two samples from each subgroup were gold-plated for surface structural study by using (Tescan, vega 3, Czech) SEM scanning electron microscope. A longitudinal cross-section of the treated tooth surface was prepared; see figure (4), to recognize the depth of the laser effect in the treated tooth.

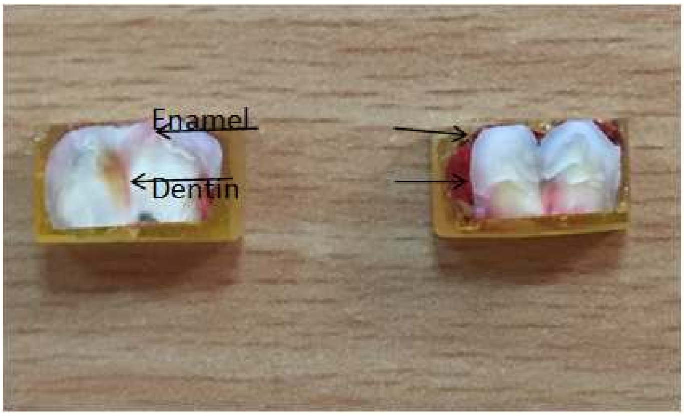

Figure 4: The Longitudinal Cross-Section of the Laser Treated Tooth Sample

The laser treated and untreated samples were characterized by an energy dispersive X-ray spectrometer. EDS; equipped with SEM (Tescan, vega 3, Czech) were employed to characterize the changes of weight percentage of elements in the enamel after laser illumination. Phosphorus and calcium ratios were determined by weight measurement, and the Ca/P ratio; of all samples, was analyzed. An artificial de-mineralization solution was prepared by mixing (0.0022 M of CaCl2, 0.05M acetic acid, 0.0022M of NaH2PO4 and a few grams of KOH for adjusting the PH to 4.5). The teeth samples were immersed in this solution at 37 o C for 48 hours. After thorough water cleaning, each laser-treated sample was weighed before and after immersion in this solution, in order to conduct the EDS analysis.

RESULTS AND DISCUSSIONS

Figure (5) presents the tooth enamel microhardness values for different number of laser pulses. The results show higher microhardness values after laser illumination. The ultimate shock wave effect was reached by many smaller shock wave components (originate from many laser pulses) added together without any probability of cracking. The maximum increase in tooth microhardness was when using 6 pulses of 100mJ Nd: YAG energy. The increase of tooth enamel microhardness after irradiation by many laser pulses is attributed to two factors: the fast laser heating and quenching by subsequent temperature gradient of a thin enamel surface layer during laser illumination, and the shockwave pressure that pushes inside when the samples are irradiated by the Nd: YAG laser. This shockwave originates from enamel surface ablation after laser energy absorption, where enamel surface experiences phase transformation from solid to vapor leading to plasma, which absorbs quickly the laser energy [17]. The plasma expands and causes a shock wave, which increases the pressure on the enamel surface[18], and as a result, increases the microhardness; i.e. lowers the tooth abrasion degree [19].

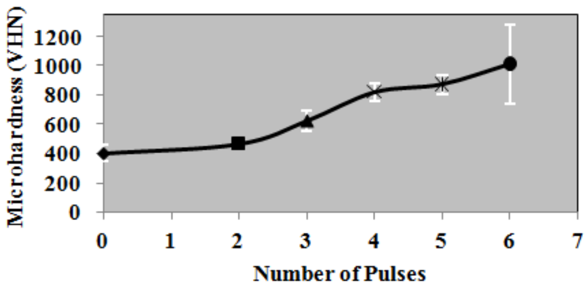

Figure 5: Tooth Enamel Microhardness Average Values with Standard Deviation Versus the Number of Laser Pulses at Fixed Laser Energy (100mJ Nd: YAG Laser)

Samples treated with laser showed moderate increased hardness (Figure 7-B) which increased when treated with combined laser and silver-nanoparticles (Figure 6-Q) as compared with control (Figure 6-A). High tooth enamel microhardness means higher resistant against tooth decay because of the reduced enamel solubility [20]. Too high tooth microhardness is not advised as the tooth becomes vulnerable to breakage at least food chewing pressure.

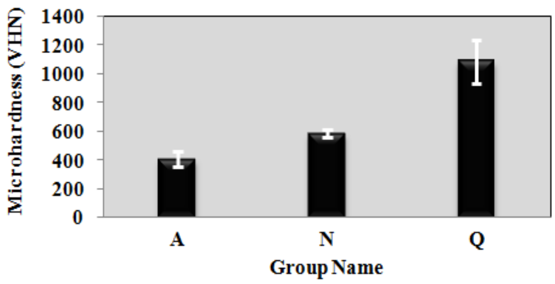

Figure 6: Tooth Enamel Microhardness Average Values for (A): Control, (N): 500mJ Nd: YAG Laser and (Q): Combined 500mJ Nd: YAG Laser with Silver Nanoparticles

Figure (7) represents the tooth surface topography images of samples' surfaces, and Figure (8) indicates the granularity distribution of the tooth samples surfaces. The figures show significant changes in the topography images and granularity distribution between the treated and the control samples.

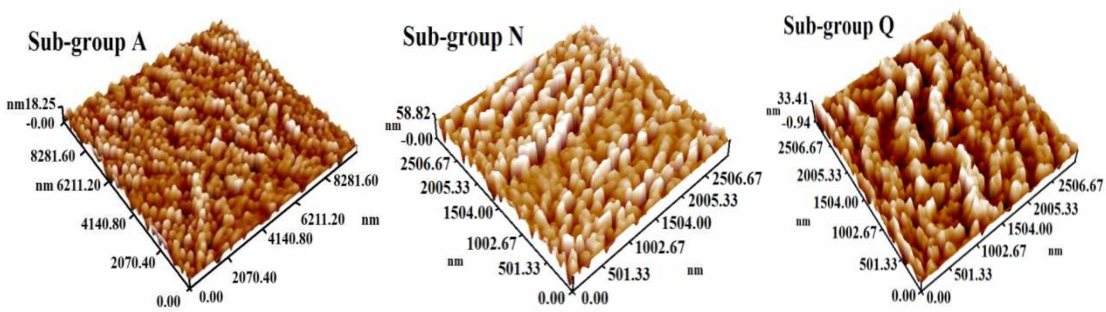

Figure 7: 3D - AFM Topographic Images of (A): Control,(N): 500mJ Nd: YAG and Q: 500mJ Nd:YAG Laser Combined with Silver Nanoparticles

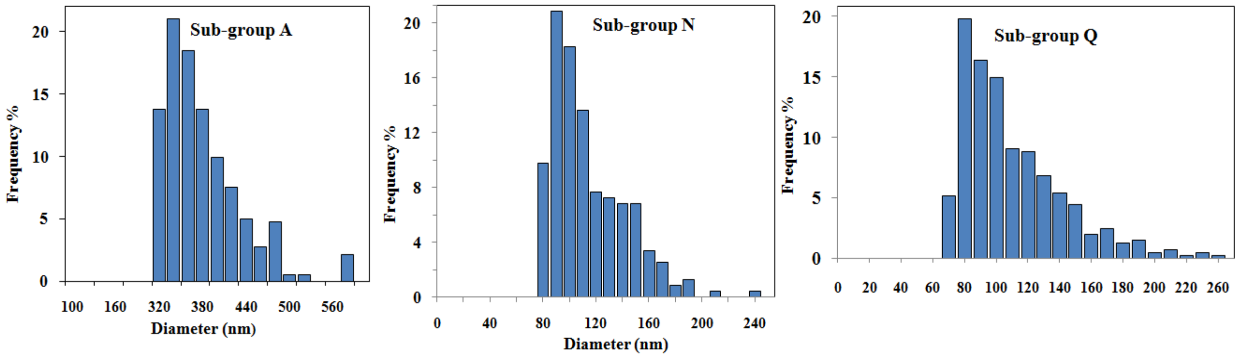

Figure 8: Granularity Accumulation Distribution Charts (A): Control. (N): Single 500mJ Nd:YAG Laser Pulse and (Q): Single 500mJ Nd:YAG Laser Combined with Silver Nanoparticles

Figure (9) demonstrates the increased roughness of the enamel surface after laser treatment with different number of laser pulses, as a result of laser induced-crystallographic changes. This increased-roughness was found useful to accommodate the silver nanoparticles on the roughened enamel surface.

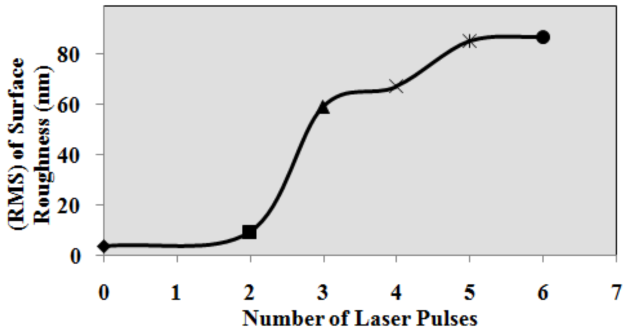

Figure 9: Enamel Surface Roughness Versus the Number of a Laser Pulses

Laser energy converted to heat in the dental hard tissue causes morphological, optical, and crystallographic changes in the dental enamel because of the thermally-induced chemical reaction; resulting from enamel heating to high temperatures. These morphologic changes may have resulted from phase transformation; caused by melting of inorganic materials, and the blockage of ion diffusion pathways [21]. Table (1) describes the changes in diameter size of enamel

grains of the samples after treating them with different number of laser pulses.

Table 1: The Grain Size of Tooth Sample at Different Treatment Conditions

Group Name	Avg. Diameter (nm)	10% Diameter (nm)	50% Diameter (nm)	90% Diameter (nm)
A (control)	390.00	0	340.00	440.00
G (2 pulses 100mJ Nd: YAG)	187.99	100.00	180.00	270.00
H (3 pulses 100mJ Nd: YAG)	304.72	240.00	300.00	360.00
I (4 pulses 100mJ Nd: YAG)	329.81	200.00	340.00	440.00
J (5 pulses) 100mJ Nd: YAG)	241.52	0	220.00	260.00
K (6 pulses 100mJ Nd: YAG)	207.12	160.00	200.00	220.00
L (5 pulses 100mJ Nd: YAG combined with silver nanoparticles)	121.35	60.00	110.00	180.00
M (5 pulses 100 mJ Nd: YAG combined with silver nanoparticles).	107.72	80.00	100.00	130.00
N (Single 500mJ Nd: YAG laser pulse)	108.60	80.00	100.00	140.00
Q (Single 500 mJ Nd:YAG laser combined with silver nanoparticles)	104.80	70.00	90.00	140.00

The enamel grain size followed a Gausian distribution with the number of laser pulses. It was 188nm at 2 laser pulses illumination but increased at 3 pulses and increased further at 4 pulses, then started to decrease at 5 and 6 laser pulses. When tooth samples were treated with combined laser and silver nanoparticles, there was a significant decrease in the enamel grain size. The enamel temperature increases with the number of laser pulses and, as a result, they change their size [22]. At first they became small but grew in size at increased temperatures [23]. The water content reduces with rising temperature and a sudden size reduction takes place when losing the amount of initially combined water in enamel [24]. The a-axis length enlargement of the enamel crystallite was found to be a function of the structurally integrated water [25]. Figure (11) shows SEM images of sub-group A (control) and other sub-groups which received a different number of laser illumination.

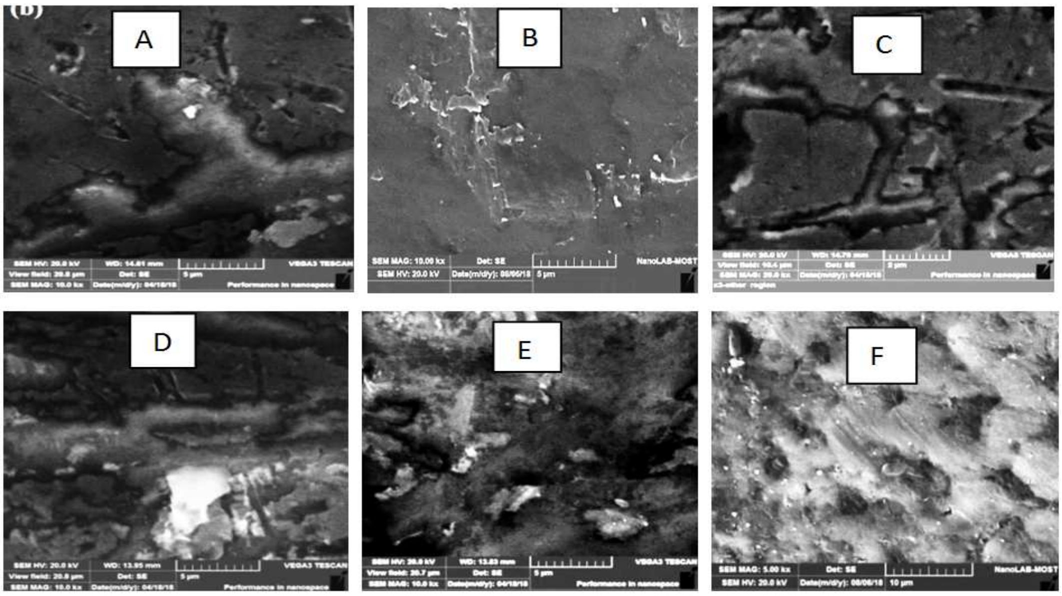

Figure 10: SEM Image of the 100mJ Laser Treated Enamel Surface of A: Control Sample, B: 3 Laser Pulses, C: 4 Laser Pulses, D: 5 Laser Pulses, E: 6 Laser Pulses and F: 5 Laser Pulses Combined with Silver Nanoparticles

Figure (11-A) represents a cross-section of the control enamel sample, and Figure (11-B) shows the cross-section of laser illuminated enamel surface by 5 laser pulses of 500mJ Nd: YAG laser. There are significant changes in the treated enamel structure compared to the control: the rods of the laser treated part are overlapping, sharper and more interconnected with each other, which explains the high microhardness and the resistance of the treated tooth against demineralization.

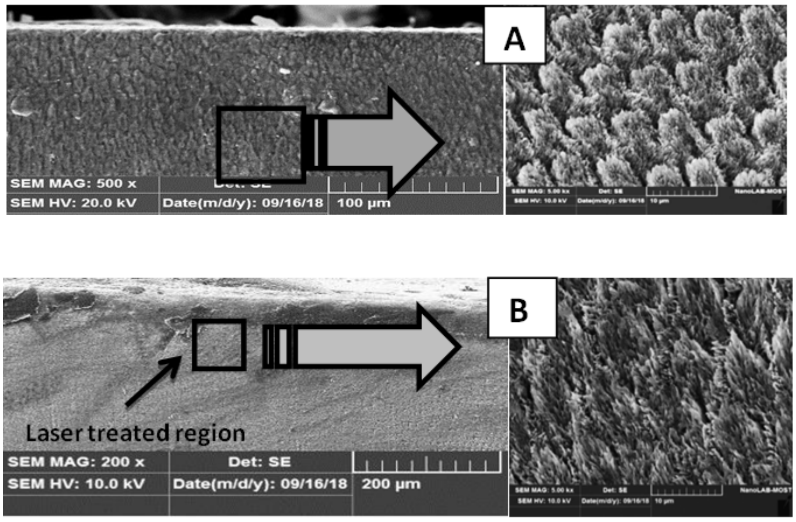

Figure 11: Longitudinal Cross-Section of A: Control and B: the Treated Tooth Enamel Sample with 5 Pulses of 400mJ Nd: YAG Laser

One tooth sample from each sub-group was individually immersed in the demineralization solution for 48 hours to test the extent of change in the calcium to phosphorus ratio. Demineralization extracts the mineral from tooth and leave spaces on the surface; and this in turn speeds enamel demineralization [21]. The demineralized samples of the laser treated sub-groups demonstrated higher Ca/P ratios than the demineralized samples of the control; see Figures (12).

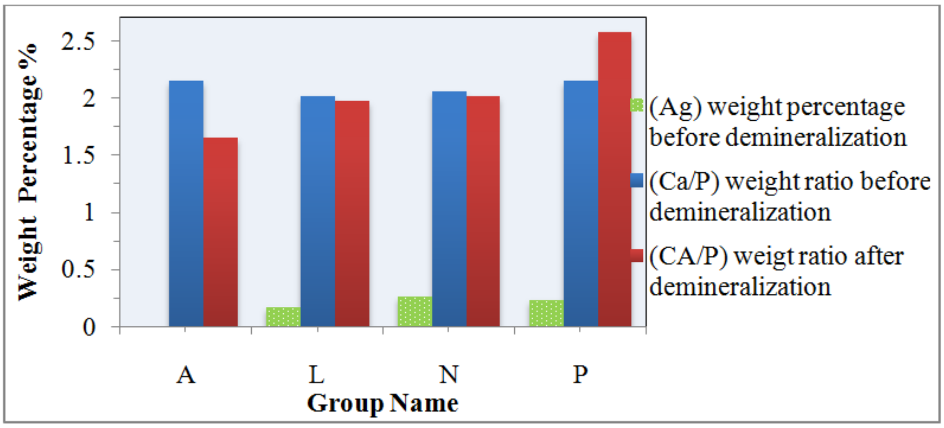

Figure 12: Calcium to Phosphorus Weight Ratio before and after the Demineralization Process, and the Nano Silver Weight Percentage of the Sub-Groups Samples; A: Control, L: Combined 5 Pulses 100mJ Nd: YAG with Silver Nanoparticles. N: Combined Single 500mJ Nd: YAG with Silver Nanoparticles. P: Single 500mJ Nd: YAG Pulse Followed by Combined Single 500mJ Nd: YAG with Silver Nanoparticles

The Ca/P ratios of (L, N, and P) samples became higher than they were before demineralization. This is likely to be resulted from silver nanoparticles on the enamel structure, which protected calcium from being dissolved during acid

interaction with teeth. The SEM images of the demineralized samples demonstrated parallel rod orientations to each other of most enamel rods and perpendicular to the tooth surface; see Figure (13), but became tangled with one another after laser treatment. The change in enamel rods orientations may decrease the acid penetration and increase the tooth resistance.

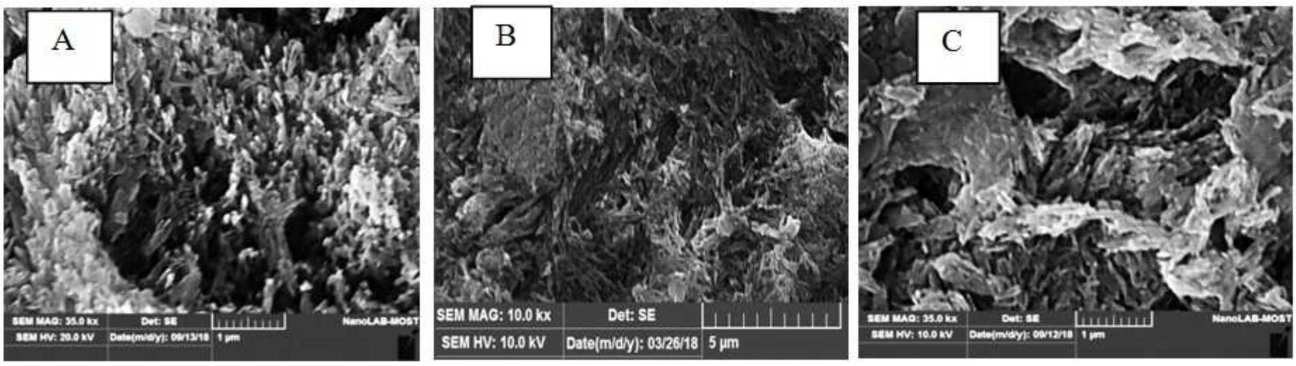

Figure 13: SEM Image of Enamel Surface after Deminerralization for 48 Hours; A: Control Sample, B: 6 Pulses of 100mJ Nd: YAG Laser, C: Combined Single 500mJ Nd: YAG Pulse with Silver Nanoparticles

CONCLUSIONS

Due to fast laser heating and quenching and to the shock wave pressure, the tooth enamel microhardness increased with the laser energy fluence from successive laser pulses. Samples treated with laser showed moderate increased hardness, but demonstrated higher hardness when treated with combined laser and silver-nanoparticles. High tooth enamel microhardness means higher resistant against tooth decay because of the reduced enamel solubility. Significant topograhic changes and granularity distribution were noticed after laser-treating the samples, with a notable increase in enamel surface roughness; due to laser induced-crystallographic changes. This increased roughness helped accommodate the silver nanoparticles on the roughened enamel surface. The enamel grain size followed a Gausian distribution with the number of laser pulses. When tooth samples were treated with combined laser and silver nanoparticles, there was a significant decrease in the enamel grain size. The enamel temperature increases with the number of laser pulses and, as a result, they changed the grain size. Starting with small size, but grew at elevated temperatures. The a-axis length enlargement of the enamel crystallite is a function of the structurally integrated water. The water content reduces with rising temperature and a sudden size reduction took place when losing the amount of initially combined water in enamel. There were significant changes in the treated enamel structure compared to the control: the rods of the laser treated part were overlapping, sharper and more interconnected with each other, which explains the high microhardness and the resistance of the treated tooth against demineralization. The demineralized laser treated samples demonstrated higher Ca/P ratios than the demineralized control samples. The Ca/P ratios became higher than they were before demineralization. This is likely to have resulted from silver nanoparticles on the enamel structure, which protected calcium from being dissolved during acid interaction with teeth. The SEM images of the demineralized control sample demonstrated parallel rod orientations which are perpendicular to the tooth surface. They became tangled with one another after laser treatment and this helped decrease the acid penetration and increase the tooth resistance.

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